originate in part from the population of alternative protein complex structures, e.g. one bound state that is in equilibrium with one or several alternative configurations. Here we present two protein complexes that exhibit fluctuations in solution: the plexin RBD:Rac1 and the EphA2:SHIP2 SAM:SAM complexes. A number of techniques are used to shift the equilibrium and/or to demonstrate the population of alternate complex configurations, including ion-pair swap mutagenesis, disulphide cross-linking and Paramagnetic Relaxation Enhancement (PRE) NMR. An ensemble approach is required to calculate the structures even when a wide collection of NMR restraints (chemical shifts, PREs, NOEs and RDCs) are available. The origin of the alternate structures is revealed by the different patterns of sidechain contacts that are possible in the complexes. These interactions and the dynamics of the complexes are further analyzed by microsecond unrestrained molecular dynamics simulations carried out on the MD optimized supercomputer Anton. The functional significance of protein complex dynamics is discussed.

## Symposium: Dynamics of G-Protein Coupled Receptors

**3094-Symp**
**Activating Mutations and Allosteric Modulators: Effects on GPCR Structure and Conformational Dynamics**
**David L. Farrens, Ph.D.**
Biochemistry and Molecular Biology, Oregon Health and Science University, Portland, OR, USA.
Like any useful switch, GPCRs are able to convert back and forth between an ''off'' (inactive) and an ''on'' (active) state. The dynamics of this process are governed by inherent aspects of a given GPCRs structure, and by interactions it may have with other molecules, such as agonist or antagonist ligands, or even other proteins. Our lab has been studying dynamic structural changes involved in GPCR activation and attenuation, primarily through use of site-directed fluorescence labeling (SDFL) methods. One SDFL method we have been developing, which we call Tryptophan Induced Quenching (TrIQ), is helpful in these studies because it can identify sites of direct fluorophore-Trp contact in proteins, and can even quantify how much of this direct contact occurs, with sub-nanosecond timescale resolution. Thus, TrIQ can be used to measure changes in the equilibrium population between two protein conformational states. In my talk, I will briefly review our TrIQ method and how we use it, primarily in studies of rhodopsin and the human cannabinoid receptor CB1. I will then review insights that TrIQ and other SDFL approaches have provided us about where helical movements occur in GPCRs, and why they enable coupling with GPCR affiliated proteins. Finally, I will discuss our recent efforts to measure the energetics involved in a key helical movement that occurs upon GPCR activation, and how a constitutively activating mutation effects the energetic of this conformational change, and discuss how allosteric ligands (both small molecule and protein) can alter GPCR signaling by effecting these movements.

**3095-Symp**
**From High-Resolution Rhodopsin Membrane Structures to Function**
**Ulrike Alexiev, Ph.D.**
Physics Department, Freie Universitaet Berlin, Berlin, Germany.
Membrane receptors are key molecules in cellular communication. The largest class of membrane receptors, the G-protein coupled receptors (GPCRs), transmits various external signals through conformational changes that lead to molecular recognition and activation of transducer molecules at the receptor surface inside the cell, subsequently triggering a signal transduction cascade.
I will present examples of conformational transitions and dynamics changes taking place upon activation and deactivation of the GPCR rhodopsin and discuss their functional relevance. Time-resolved fluorescence and polarization methods were employed to follow the conformational transitions. Modulation of structural dynamics, proceeding in the pico- and nanosecond time domain, by protonation changes of the receptor-ligand complex in the microseconds to seconds domain is traced in pump-probe experiments, which combine time-resolved measurements on these two vastly different time scales. This method allows us to obtain dynamic information of both the receptor and transducer or regulatory molecules during the different stages of their engagement, information that is not possible to extract from static X-ray structures. Single molecule total internal reflection fluorescence (TIRF) microscopy experiments for inter- and intramolecular dynamics and fluorescence lifetime imaging microscopy (FLIM) extend the studies of receptor dynamics to the cellular level.

**3096-Symp**
**Probing GPCR Signaling with Genetically-Encoded Non-Natural Amino Acids**
**Thomas P. Sakmar**.
The Rockefeller University, New York, NY, USA.
We are interested in uncovering the principles that underlie ligand recognition in heptahelical G protein-coupled receptors (GPCRs) and to understand with chemical precision how receptors change conformation in the membrane bilayer when ligands bind. We have developed an interdisciplinary approach that employs a number of new converging technologies: i) all atom and coarse grain molecular dynamics (MD) computer simulations of GPCRs in membrane bilayers in concert with experimental validation, ii) unnatural amino acid mutagenesis of GPCRs using amber codon suppression technology, iii) interrogation of receptor dynamics using advanced FTIR (Fourier-transform infrared spectroscopy) and solid state NMR methods, iv) use of nanoscale apolipoprotein bound bilayers (NABBs) as membrane mimic support structures for GPCRs. Our near-term aim is to employ single-molecule detection (SMD) of GPCRs by TIRF (total-internal reflectance fluorescence) microscopy in self-assembling oriented tethered bilayers or in NABBs using microfluidics. This talk will focus on unnatural amino acid mutagenesis as a tool for targeted photocrosslinking methods and bioorthogonal labelling of heptahelical receptors in live cells.

**3097-Symp**
**Structural Insights into the Dynamic Process of G Protein Coupled Receptor Activation**
**Brian Kobilka**.
Stanford University, Stanford, CA, USA.
Research in my lab is directed at understanding the structural basis for the functional properties of G protein coupled receptors (GPCRs), which constitute the largest family of membrane proteins in the human genome. GPCRs conduct the majority of transmembrane responses to hormones and neurotransmitters, and mediate the senses of sight, smell and taste. The β2AR adrenoceptor (β2AR) is a prototypical Family A GPCR that mediates physiologic responses to adrenaline and noradrenaline. It regulates the activity of several distinct signaling pathways through both G protein dependent and G protein independent mechanisms. Like many GPCRs that respond to hormones and neurotransmitters, the β2AR exhibits modest basal activity in the absence of an agonist. This activity can be modulated by a spectrum of synthetic ligands ranging from inverse agonists, which suppress basal activity, to full agonists. We have obtained three-dimensional structures of the β2AR in inactive and active conformations, as well as a structure of the β2AR in complex with the G protein Gs. We have also used fluorescence, EPR and NMR spectroscopy to study the dynamic properties of the receptor, and to map ligand-specific conformational changes. I will discuss what we these studies have taught us about the structural basis of β2AR function.

## Symposium: Dynamics and Localization of RNAs

**3098-Symp**
**Following Single mRNAs from Birth to Death in Living Cells**
**Robert H. Singer**, Timothee Lionnet, Hye Yoon Park, Bin Wu, Tatjana Trcek, Sami Hocine, Daniel Larson.
Albert Einstein Coll Med, Bronx, NY, USA.
Live cell imaging has been instrumental in analyzing the dynamic properties of RNA. New technologies in optical microscopy and fluorescent probe development have been pushing the envelope of our analysis capabilities. We have been dedicated to developing and implementing these technologies to further the understanding of single mRNA dynamics in cells and organisms. We have utilized computational approaches to analyze real-time transcription activities of endogenous genes from yeast to human cells. We have employed a plethora of imaging methods, ranging from confocal and multiphoton microscopy, long-term cell imaging, high-speed real-time widefield microscopy, single molecule tracking, and we have developed super-registration microscopy and fluorescence fluctuation analysis. We have investigated key processes of RNA synthesis: initiation, elongation, termination, as well as nuclear pore export, cytoplasmic trafficking, localization and decay. Mathematical modeling